

**EXAMPLE 52****NMR experiments on modified oligonucleotides comparison of 3',5' versus 2',5' internucleotide linkages and 2'-substituents versus 3'-substituents by NMR**

[0190] The 400MHz  $^1\text{H}$  spectrum of oligomer d(GAU<sub>2</sub>\*CT), where U<sub>2</sub>\*= 2'-O-aminohexyluridine showed 8 signals between 7.5 and 9.0 ppm corresponding to the 8 aromatic protons. In addition, the anomeric proton of U\* appears as a doublet at 5.9 ppm with J<sub>1',2'</sub>= 7.5Hz, indicative of C2'-endo sugar puckering. The corresponding 2'-5' linked isomer shows a similar structure with J<sub>1',2'</sub>= 3.85 Hz at 5.75 ppm, indicating an RNA type sugar puckering at the novel modification site favorable for hybridization to an mRNA target. The proton spectrum of the oligomer d(GACU<sub>3</sub>\*), where U<sub>3</sub>\*=3'-O-hexamine, showed the expected 7 aromatic proton signals between 7.5 and 9.0 ppm and the anomeric proton doublet at 5.9 ppm with J<sub>1',2'</sub>= 4.4 Hz. This suggests more of a C3'-endo puckering for the 3'-O-alkylamino compounds compared to their 2' analogs.  $^{31}\text{P}$  NMR of these oligonucleotides showed the expected 4 and 3 signals from the internucleotide phosphate linkages for d(GAU\*CT) and d(GACU\*), respectively. 3'-5' Linked vs. 2'-5' linked have different retention times in RP HPLC and hence different lipophilicities, implying potentially different extent of interactions with cell membranes.

**EXAMPLE 53****T<sub>m</sub> Analysis of 2',5'-linked oligonucleotides versus 3',5'-linked oligonucleotides**

[0191] Thermal melts were done as per standarized literature procedures. Oligonucleotide identity is as follows:

Oligonucleotide A is a normal 3'-5' linked phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU\* CTG CG) SEQ ID NO: 14 where the \* indicates the attachment site of a 2'-aminolinker. Oligonucleotide B is a normal 3'-5' linked phosphodiester oligoribonucleotide of the sequence d(GGC TGU\* CTG CG) SEQ ID NO: 14 where the \* indicates the attachment site of a 2'-aminolinker. Each of the ribonucleotides of the oligonucleotide, except the one bearing the \* substituent, are 2'-O-methyl ribonucleotides. Oligonucleotide C has 2'-5' linkage at the \* position in addition to a 3'-aminolinker at this site. The remainder of the oligonucleotide is a phosphodiester oligodeoxyribonucleotide of the sequence d(GGC TGU\*

CTG CG) SEQ ID NO: 14. The base oligonucleotide (no 2'-aminolinker) was not included in the study.

Table IIIa

OLIGONUCLEOTIDE	MODIFICATION	DNA TARGET	RNA TARGET
A	none	52.2	54.1
	2'-aminolinker	50.9	55.5
B	none	51.5	72.3
	2'-aminolinker	49.8	69.3
C	none	NA	
	3'-aminolinker	42.7	51.7

[0192] The 2'-5' linkages demonstrated a higher melting temperature against an RNA target compared to a DNA target.

#### EXAMPLE 54

#### Snake Venom Phosphodiesterase and Liver Homogenate Experiments on Oligonucleotide Stability

[0193] The following oligonucleotides were synthesized following the procedure of Example 49.

Table IV  
Modified Oligonucleotides  
synthesized to evaluate stability

SEQ ID (ISIS) # NO. #	Sequence (5'-3')	Backbone	Chemistry
15 (22110)	TTT-TTT-TTT-TTT-TTT-T*T*T'-T*	P=O	3'-O-MOE
16 (22111)	TTT-TTT-TTT-TTT-TTT-T*T*T'-U*	P=O	3'-O-MOE
15 (22112)	TTT-TTT-TTT-TTT-TTT-T*T*T'-T*	P=S	3'-O-MOE
16 (22113)	TTT-TTT-TTT-TTT-TTT-T*T*T'-U*	P=S	3'-O-MOE

15	(22114)	TTT-TTT-TTT-TTT-TTT <sub>o</sub> -T' <sub>o</sub> T' <sub>o</sub> T' <sub>o</sub> T'	P=S/P=O	3'-O-MOE
16	(22115)	TTT-TTT-TTT-TTT-TTT <sub>o</sub> -T' <sub>o</sub> T' <sub>o</sub> T' <sub>o</sub> U*	P=S/P=O	3'-O-MOE

[0194] <sup>1</sup>All nucleosides with an asterisk contain 3'-O-(2-methoxyethyl). All nucleosides with a # contain 2'-O-(2-methoxyethyl).

The oligonucleotides were purified following the procedure of Example 50 and analyzed for their structure.

Table V  
Properties of Modified Oligonucleotides

SEQ ID NO. #	(ISIS) #	#Sequence (5'-3') <sup>1</sup>	Expected Mass	Observed Mass	HPLC <sup>2</sup> T <sub>R</sub>	#Ods(260nm) Purified (min.)
15	(22110)	TTT-TTT-TTT-TTT-TTT-T' T' T' -T'	6314.189	6315.880	20.39	174
16	(22111)	TTT-TTT-TTT-TTT-TTT-T' T' T' -U*	6004.777	5997.490	20.89	147
15	(22112)	TTT-TTT-TTT-TTT-TTT-T' T' T' -T'	6298.799	6301.730	25.92	224
16	(22113)	TTT-TTT-TTT-TTT-TTT-T' T' T' -U*	6288.745	6286.940	24.77	209
15	(22114)	TTT-TTT-TTT-TTT-TTT <sub>o</sub> -T' <sub>o</sub> T' <sub>o</sub> T' <sub>o</sub> T'	6234.799	6237.150	24.84	196
16	(22115)	TTT-TTT-TTT-TTT-TTT <sub>o</sub> -T' <sub>o</sub> T' <sub>o</sub> T' <sub>o</sub> U*	6224.745	6223.780	23.30	340

[0195] <sup>1</sup>All nucleosides with an asterisk contain 3'-O-(2-methoxyethyl). All nucleosides with a # contain 2'-O-(2-methoxy) ethyl. <sup>2</sup>Conditions: Waters 600E with detector 991; Waters C4 column (3.9X300mm); Solvent A: 50 mM TEA-Ac, pH 7.0; B: 100% acetonitrile; 1.5 mL/min. flow rate; Gradient: 5% B for first five minutes with linear increase in B to 60% during the next 55 minutes.

## EXAMPLE 55

### 3'-O-Aminopropyl modified oligonucleotides

[0196] Following the procedures illustrated above for the synthesis of oligonucleotides, modified 3'-amidites were used in addition to conventional amidites to prepare the oligonucleotides listed in tables VI and VII. Nucleosides used include: N6-benzoyl-3'-O-propylphthalimido-A-2'-amidite, 2'-O-propylphthaloyl-A-3'-amidite, 2'-O-methoxyethyl-